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# THE NUTRITIONAL GOLDMINE WASTE: THE SPENT PUPAE OF MULBERRY, ERI AND OAK TASAR SILKWORMS FOR COMBATING MALNUTRITION

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***ABSTRACT***

***With a view to provide the utility of edible silkworm pupae waste in a better way, comparative nutritional profiling of both unspent and spent pupa of three different species of silkworm, Bombyx mori, Samia cynthia ricini and Antheraea proylei were evaluated. The nutritional profiling of A. proylei pupa, a native non-mulberry silkworm species of Manipur is reported for the first time. The protein content of spent pupae was highest (41%) in the non- mulberry species and S. c. ricinifollowed by A. proylei (40%) and B. mori (31%). Both spent and unspent silkworm pupae yielded good sources of protein. The crude fat content of unspent and spent pupae ranged from 12 -17%. The silkworm also has considerable macro- nutrient content. Hence, utilization of spent pupa waste towards product development with value addition are the need of the hour.***

***KEYWORDS: Edible Insect, Nutritional Values, Silkworm, Spent Pupa & Unspent Pupa***

**Original Article**

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## INTRODUCTION

Silkworm pupa occupied one of the major groups among 100 species of edible insect consumed by the indigenous peoples of northeast India [1,2]. Over 40 different ethnic groups consumed five traded species of silkworms viz. *Samia cynthia ricini* (eri), *Bombyx mori*(mulberry), *Antheraea assamensis* (muga), *A. proylei*(tasar) and *A. pernyi*(tasar)[3,4]. In Assam, among the silkworm species, consumption of eri was highest (87.7%), followed by muga (57.4%) and mulberry (24.6%)[5]. In North East India, silkworms are reared both for consumption purposed as well astrading. In various local markets of northeast India such as Nagaland, Assam etc. value added well packed pickle form of eri pupae are sold about Rs. 150 to 200/ 200mg (@ $ 14.7/ Kg, where $1=Rs. 68)and dry waste pupae are sold approximately Rs. 20-60/Kg ($ 0. 29 to 0.87/Kg) for utilization in fish feed[6]. Enormous volume of silkworm pupa is generated as a major by-product in sericulture industry. This by-product which is presently felt as wastes can be put into better use by converting value-based products, thereby making the industry more profitable and economically viable spot. The complete biomass utilization of silkworm industry can certainly make the sericulture more profitable[7-9]. The cost of the silk can be proportionately brought down in sericulture industry by converting seri-wastes into useful products. 70 % of the silkworm cocoon biomass is discarded as pupa waste in sericulture industry. Profitable conversion of by-products to high value utilities through phytoand

post-harvest technologies[10], can reduced the production cost, pollution and maximize utilization of resources to cater the ever-growing population and their demanding wants.

The indigenous people of northeast India recognised the importance of this edible insect from the nutritional point of view too. To reveal the utility of waste silkworm pupa in preparation of human or animal food supplement, nutritional profiling of both unspent and spent pupa of three different species, *B. mori* (mulberry), *S. c. ricini* (eri) and *A. proylei* (Oak tasar) were evaluated and compared. The nutritional profiling of mulberry and eri pupae were reported earlier [5,11,1] but comparative nutritional evaluation of unspent (unprocessed) and spent (processed) pupa was not reported yet. Further, nutritional profile of the oak tasar silkworm pupa, *A. proylei*, a native non- mulberry silkworm of Manipur is reported for the first time. Therefore, the present study aims to determine and asses the variation of nutritional value between processed and unprocessed pupa of the three species of silkworm, *B. mori* (mulberry), *S. c. ricini* (eri) and *A. proylei* (Oak tasar).

## MATERIALS AND METHODS

### Insect Material

Theunspent and spent pupae were collected from six sericulture firms located in and around Imphal West and Imphal East districts of Manipur. The pupae were thoroughly washed and peeled off the thin covering from the spent pupae. Five batches were maintained for each species for both spent and unspent pupae. Each batch contained about thousand pupae and was dried in oven at 55°C until a stable weight is obtained for all species. Dried pupae were crushed separately into fine powder for each replication. It was then processed for further biochemical analysis. Required quantity was used for each replication according to the provided protocols.

### Proximate Compositions

Proximate compositions (moisture content, crude protein, crude fat, crude fibre, ash) were determined according to the Association of Official Analytical Chemists methods [12](AOAC, 1990).

### Protein

Protein was indirectly determined from the amount of nitrogen produced[13,14.]50 mg oven dried samples was digested in 1ml of conc. Sulphuric acid (containing 5% salicylic acid w/v). It was heated on a hot plate in a fumigation chamber until colour becomes dark and fumes appear. Then sample heating again for 5min after addition of 20mg sodium thiosulphate and cooled at room temperature, 1ml of 60% perchloric acid containing 1mg/ml of Copper(II) sulfate pentahydrate was added. The heating is continued for 10-15min till the black colour disappears to transparent liquid. Then, the sample was cooled and diluted upto 100ml with distilled water. Further, the non-protein nitrogen content was extracted from total nitrogen in order to estimate the crude protein content. The non-protein nitrogen was extracted with ice-cold 10%TCA. The protein content were estimated by conversion of nitrogen percentage to protein by adopting the formula, Protein % = N% x conversion factor (6.25).

### Carbohydrate

Carbohydrate content was determined by Anthrone method[15]by taking glucose as standard.100 mg of insect sample was hydrolyzed by keeping it in boiling water bah for 3h with 5ml of 2.5ml of 2.5N HCl and cool to room temperature. It was neutralized with sodium carbonate until the effervescence ceases. Further the content was centrifuged and supernatant was used for estimation of carbohydrate. The supernatant was allowed to react with anthrone reagent to

form a dark green colour solution for which optical density was read at 630nm.

**Fat**

Total crude fat was evaluated by using chloroform-methanol mixture[16]. 500mg of sample was homogenised with petroleum ether. It is filtered to previously weighed beaker. After four to five times of such repeated extraction, the filtrate is placed in an oven at $50^0$ C for complete evaporation. The extract is allowed to cool at room temperature and reweighed. The difference in the weight gives the amount of fat present.

**Ash**

The ash content was estimated by using the muffle furnace (NSW Model-103).2 gm of each sample were weighed in a silica crucible and heated for six hours at600°Cin muffle furnace at $600^oC$. After cooling in a desiccator in room temperature, greyishash are weighed and calculated.

**Fibre**

The crude fibre content was estimated by dilute acid and alkali hydrolysis method [12].200 mL of 0.25 N sulphuric acid solution was heated for 30 minute with two grams of fat-free, powdery form of each sample and filtered through a Buchner funnel. After proper washing with distilled water, the residue was boiled with 200 mL of 0.313 N sodium hydroxide for 30 min and washed again with 10% HCl and twice with ethanol. The residue was dried at 100°C in an oven overnight. It was ignited in at 500° C in a muffle furnace for three hours to obtain the weight of the ash.

**Energy**

1gm of the sample was ignited electrically in Digital bomb calorimeter (model RSB-3/5/6/6A, Rajdhani Scientific Instrument) to estimate the energy content for the energy estimation, the heat released during the complete oxidation of the compound was measured through the temperature change in the water bath surrounding the bomb by a digital sensor. The heat of combustion at a constant volume was calculated from the resulting rise in temperature.

**Minerals**

Mineral elements such as iron (Fe), calcium (Ca), zinc (Zn), copper (Cu), magnesium (Mg), sodium (Na) and potassium (K) were estimated using atomic absorption spectrophotometer (AAS) (Perkin Elmer AAS-200/Analyst Version – 6, USA). 500 mg of dried powder of pupae was digested with 10:4: 1 (HNO3: HClO4: H2SO4) in Kel-Plus digestion system (Pelican, India). The digested sample was diluted suitably with sterile double distilled water and filtered with whatman filter paper. Using appropriate multi-element hollow cathode lamps (Lumina Lamp, Perkin Elmer), mineral elements were analysed against a standard reference solution for AAS (Accutrace Reference Standard, USA)[17].

**Antioxidant**

The methanol extract of silkworm pupae was analyzed for antioxidant property on the basis of their scavenging activity of stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radicals [18]. Ascorbic acid was used as the standard, and absorbance was measured at 517 nm. The $IC_{50}$ value denotes the concentration of the sample required to scavenge 50% of the DPPH free radicals. Relatively stable organic free radical, DPPH has been widely used in the determination of antioxidant activity of extract [19]and was chosen for the study.

**Statistical Analysis**

One way ANOVA was used to test the significant difference among mean values for proximate composition and minerals values of three species of silkworm pupae for both spent and unspent pupae. Modified t-test was used to compare the significant different between unspent and spent silkworm pupa. A significance level of 0.05 was used to reject the null hypothesis. Statistica Version10 (Stat Soft, www.statsoft.com) was used to analyzed the data.

## RESULTS AND DISCUSSIONS

Manipur has a unique identity for producing all the three traded varieties of silkworm viz., mulberry, eri and temperate oak tasar. The average waste of silkworm pupae produced yearly in the state was determined based on the vanya silk production reported by Central Silk Board and depicted in table 1. In all the three species, about 80-90% of the silk cocoon is produced as pupal waste. The recovery of pupal waste from the total raw silk production of India can be utilized for animal feed production as it fulfils the amino acid requirement [20].

**Proximate Composition**

The protein, present in both non-mulberry and mulberry pupae can be utilized as supplementary food for malnourished children with the preparation of value added products. The nutrient properties of unspent and spentpupae of three silkworm species are given in table 2. The overall protein content of spent pupae was highest (41%) in the non-mulberry species, *S. c. ricini*followed by *A. proylei* (40%) and *B. mori* (31%). There was significant variation in the protein content of pupae at 5% level among the three species (Table 2). T-test analysis of protein content between unspent and spent pupae revealed no significant difference for all the three species. In eri silkworm prepupae and pupae, protein content was reported as 16% whereas in the defatted pupae meal, it was as high as 75 % [1] and 54% on dry weight basis [3]. In *B. mori* chrysalis toasts, it was ranging from 49.1-53.5% [21]. Generally, both spent and unspent silkworm pupa yielded good sources of protein. High protein content in other edible insect, *Crocothemes servillia* (70 %) was also reported [22]. The high protein in silkworm pupae could offer immense potential for mitigating protein deficiency in a developing country such as India[11]. The crude fat content of unspent pupae ranged from 12% in *A. proylei* to 17 % in *B. mori* whereas, 12 to 17% in spent pupae (Table.1). A significant difference was observed in the fat content between the mulberry (*B. mori*) and non-mulberry (*A. proylei* and *S. c. ricini*) at 5% level of ANOVA whereas t-test revealed no significant difference between unspent and spent pupa in all the three species. High fat content in pupae contributes significant source of oil for diet. 11.09 to 20.01% of fat content was reported in silkworm pupae of eri, muga and mulberry [1,10]. The fatty acid of edible insects is different from other animal in having higher content [23]. Estimation of lipid is considered as one of the key factors for nutritional evaluation of any material[24]. The ash content of the spent pupae ranged from 1 to 3 % in eri and mulberry silkworm[11] and resulted low in ash content too. The spent silkworm pupae showed low carbohydrate (1 to 2 %) content. Low content in carbohydrate (1 to 2 %) was also reported in earlier works on muga and eri [11,5]. It also revealed low in fibre content ranging from 1 to 2 % in both unspent and spent pupae. Further, it was observed that there was no marked difference in the amount of energy given by the unspent and spent silkworm pupae. However, there is a significant differencebetween the energy content among different silkworm species (Table 1). The spent pupa of *B. mori* yielded the highest energy, 653 Kcal/100gm.

**Minerals Element**

The mineral profiles of the unspent and spent silkworm pupae of three species are presented in Table 3. Silkworm pupae have an appreciable amount of minerals such as calcium, iron, zinc and magnesium. There is a significant difference in iron content within the three species ($p \leq 0.05$). However, Iron content was found to be the highest (111 mg/100gm in unspent pupa and 96 mg/100gm in spent pupa) in *S.c.ricini*as compare to the other two species. Magnesium content was observed to be highest in *S.c.ricini* with an amount of 622 (mg/100gm). There was no significant difference among the other minerals content except for iron and sodium between unspent and spent pupae as indicated by the t-test. Hence, both unspent and spent silkworm pupae can provide the required nutritive minerals for human/animal body functions. It could consumed along with other food to complement the nutrient supplement in human/animal diet.

Minerals played important metabolic and physiologic roles in living system. Iron, zinc, copper and manganese strengthen the immune system as antioxidant enzyme cofactors[25]. Minerals like magnesium, zinc and selenium prevent cardiomyopathy, muscle degeneration, growth retardation, impaired spermatogenesis, immune-logic dysfunction and bleeding disorder[26]. Edible insects possess good nutritional content of minerals such as iron, zinc, potassium, sodium, calcium, phosphorus, magnesium, manganese and copper[27]. It was observed from the present results that silkworm pupae, either in spent or unspent form, are good source of minerals. Iron content is found to be highest (111 mg/100gm in unspent pupa and 96 mg/100gm in spent pupa) in *S. c. ricini* as compare to the other two species. Presence of good amount of iron in other edible insects has also been emphasized in earlier works [28, 29]. Iron deficiency is a major problem in women's diets in developing world, particularly among pregnant women [30], so consumption of silkworm pupa will be useful in their health. Magnesium is needed for more than 300 biochemical reactions in the body. It helps to maintain normal muscle and nerve function, keeps heart rhythm steady, supports a healthy immune blood and regulates blood sugar levels [31]. Good amount of magnesium also observed, the highest being in *S. c. ricini* with 622 mg/100gm. Eri pupa also content good amount of zinc (19 mg/100gm) likewise in other edible insects such as mopane caterpillar (14mg/100gm) and larva of palm weevil, *Rhynchophorus phoenicis* (26 mg/100gm)[32].

**Antioxidant property**

In the present study, DPPH free radical scavenging assay of methanol extracts of three species of silkworm pupae (sericulture waste) were analysed and presented in the Figure 1. DPPH•, a stable free radical with characteristics absorption at 517 nm in methanol was used to study the radical scavenging effects of the Methanolic pupal extract of three silk worm pupae. As antioxidants donate proton to this radical, the absorption of scavenging activity of DPPH decreases. The decrease in the absorbance at 517 nm is taken as a measure of the extent of radical scavenging. The antioxidant property was assessed for spent pupa only, as there was no significant difference revealed in the nutritive content of unspent and spent pupae. The methanol extract of *S. c. ricini*pupa obtained $IC_{50}$ %at 68µg/ml, indicating good antioxidant activity among the three species. However, $IC_{50}$ % of *B.mori* and *A. proylei* showed 510 and 500µg/ml respectively indicating weaker or nil antioxidant activity. Presence of anti-oxidant property indicates a good sign of food with the ability to have the reductive capacity and free radical scavenging activities in various mechanisms of the body for good health. In addition to the potential nutritional profile, antioxidant property of silkworm pupae was determined. Among the three-silkworm species, eri pupae possess considerable value of antioxidant property like other plant product. The eri silkworm pupae with $IC_{50}$ % of 68µg/ml was found to be lower than the potential antioxidant property of the plant extract, *Calotropis procera* (121.25 µg/ml) [33]. Possessing of good antioxidant property in muga and eri species had already been observed

and reported [23]. The reducing power of a compound is related to its electron transfer ability and may, therefore serves as a significant indicator of its antioxidant activity [34]. The phenolic compounds were directly correlated with its antioxidant ability. The physiological effects of flavonoids include possible antioxidant activity, therefore, suggesting their role in prevention of coronary heart diseases including atherosclerosis [35].

## CONCLUSIONS

The commercially traded sericegenous insect viz. the mulberry, eri, tasar and muga would be term as Goldmine species for being the sources of the topmost quality fabric, the queen fabric and also supporting livelihood and legacy starting from poorest to riches since time immemorial. The pupae, the Goldmine waste, for being perfect nutritive sources for malnutrition supplement and delicacies. Silkworm pupa is treated as one of the important food items among edible insects by the indigenous people, who have the tradition of entomophagy in their culture. They consumed silkworm pupa for its taste, flavour and culture without proper knowledge of its high nutritive value. On the other side, a large volume of silkworm pupae is discarded from sericulture industry as seri-waste. Therefore, pupae offer an immense scope for preparation of pupa protein concentrate of good quality protein that can be used in animal nutrition, especially for the pet food industry or poultry feed, thereby generating additional income in the silk industry. The study also clearly suggested that both spent and unspent pupal waste generated from sericulture farms can gain extra income without compromising its nutritional value. Hence, in-depth research towards product development, quality optimization and value addition through application of suitable technology are the need of the hour, so as to exploit fully the larger biomass of sericulture, the pupal waste, for future generation.

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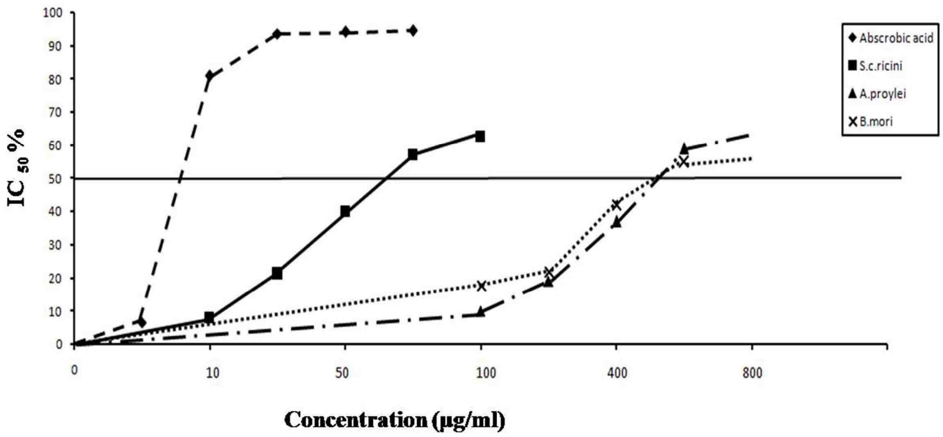


**Figure 1: DPPH Scavenging Assay of Three Species of Silkworm Pupa Compared with Standard Ascorbic Acid (IC $_{50}$ % vs Concentration)**

**Table 1: Average Amount of Waste Pupa Generated Annually After Silk Yarn Production from the Three Silkworms Species**

| Species | Production of Vanya Silk (MT) | Production of Cocoon (MT) | Pupal Waste (MT) | Waste Generated (%) |
|---|---|---|---|---|
| *B. mori* | 115 | 897 | 759.49 | 84.67 |
| *A. proylei* | 3.09 | 38.12 | 34.03 | 90.10 |
| *S. c. ricini* | 300.75 | 4973.65 | 4060.12 | 81.63 |

MT=Metric tons

**Table 2: Proximate Composition of Three Different Species of Silkworm Pupa**

| Nutritional parameters | Stage of pupa | *B. mori* | *A. proylei* | *S. c. ricini* |
|---|---|---|---|---|
| Moisture (%) | unspent | 49.16±0.17$^{b}$ | 47.85±0.51$^{b}$ | 44.24±0.43$^{a}$ |
| | spent | 48.11±0.18$^{c}$ | 46.73±0.11$^{b}$ | 43.39±0.29$^{a}$ |
| Protein (%) | unspent | 29.09±1.55$^{a}$ | 39.44±0.49$^{b}$ | 40.95±2.20$^{b}$ |
| | spent | 31.04±1.05$^{a}$ | 40.14±1.40$^{b}$ | 41.15±1.12$^{b}$ |
| Fat (%) | unspent | 17.34±0.19$^{b}$ | 12.26±0.60$^{a}$ | 12.96±0.57$^{a}$ |
| | spent | 16.75±0.46$^{b}$ | 11.55 ±0.56 $^{a}$ | 12.67±0.32$^{a}$ |
| Carbohydrate (%) | unspent | 1.76±0.08 $^{c}$ | 2.09±0.07$^{b}$ | 1.22±0.01$^{a}$ |
| | spent | 1.01±0.07 $^{b}$ | 1.74±0.19$^{a}$ | 1.08±0.03 $^{a}$ |
| Ash (%) | unspent | 1.37±0.07 $^{b}$ | 1.92±0.04$^{a}$ | 1.12±0.03 $^{a}$ |
| | spent | 3.25±0.08$^{b}$ | 1.85±0.05$^{a}$ | 1.07±0.04 $^{a}$ |
| Crude fibre (%) | unspent | 1.91±0.06 $^{a}$ | 1.92±0.04$^{b}$ | 1.12±0.11 $^{b}$ |
| | spent | 1.70±0.12 $^{a}$ | 1.58±0.30$^{b}$ | 1.35±0.11$^{b}$ |
| Energy (Kcal/100gm) | unspent | 649.75 ± 4.04$^{c}$ | 583.21± 4.61$^{a}$ | 602.84± 4.50$^{b}$ |
| | spent | 653.42±13.09$^{b}$ | 557.58 ±8.27$^{a}$ | 647.39±9.15$^{b}$ |
| The superscript alphabet along the mean values (±SE) determined significant difference among the species ($p \leq 0.05$). | | | | |

**Table 3: Mineral Content of Unspent and Spent Pupa of Three Different Silkworm Species**

| Mineral (mg/100 g) | Pupa type | *B. mori* | *A. proylei* | *S. c. ricini* |
|---|---|---|---|---|
| Iron | unspent | 12.68 ± 0.58$^{a}$ | 34.24 ±1.91$^{b}$ | 110.98 ±5.61 $^{c}$ |
| | spent | 10.57± 0.44$^{a}$ | 26.25±0.66$^{b}$ | 96.12±3.23$^{c}$ |
| Calcium | unspent | 32.92 ± 1.02$^{b}$ | 36.27 ± 0.42$^{c}$ | 25.33 ± 0.33$^{a}$ |
| | spent | 30.51 ± 0.42$^{c}$ | 22.35 ± 0.78 $^{a}$ | 25.2 ± 0.83$^{b}$ |
| Zinc | unspent | 19.15 ± 0.35$^{a}$ | 24.26 ± 0.38 $^{b}$ | 25.2 ± 0.83$^{b}$ |
| | spent | 16.51 ± 0.27$^{a}$ | 17.80±1.76 $^{b}$ | 19.12±1.01$^{b}$ |
| Copper | unspent | 1.74 ± 0.08$^{ab}$ | 2.29± 0.11$^{b}$ | 1.10 ± 0.01$^{a}$ |
| | spent | 1.19±0.03 $^{a}$ | 1.95 ± 0.05$^{b}$ | 1.21±0.04 $^{ab}$ |
| Magnesium | unspent | 379.07±15.31$^{b}$ | 371.22±19.78$^{a}$ | 621.75±2.25$^{c}$ |
| | spent | 386.40± 21.70$^{b}$ | 365.75 ±30.91$^{a}$ | 612±58.0 $^{c}$ |
| Sodium | unspent | 1.25± 0.07$^{b}$ | 1.10 ±0.01$^{a}$ | 1.17 ± 0.01$^{b}$ |
| | spent | 1.07±0.02 $^{b}$ | 1.39±0.01$^{b}$ | 0.91±0.19$^{a}$ |
| Potassium | unspent | 2.45 ± 0.02 $^{b}$ | 2.74 ± 0.18$^{b}$ | 2.29 ±0.12$^{a}$ |
| | spent | 1.55±0.12$^{b}$ | 2.30 ±0.1 $^{c}$ | 1.37 ± 0.03$^{a}$ |
| The superscript alphabet along the mean values (±SE) determined significant difference among the species ($p \leq 0.05$). | | | | |